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ADRENAL SECRETION AND PLASMA CBG LEVELS IN THE IMMATURE MALE RAT: EFFECTS OF 5α REDUCED ANDROGENS AND ANTIANDROGENS

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SUMMARY

The present study describes the effects of the 5α reduced androgens dihydrotestosterone propionate (DHTP) and 5α androstan-3α,17β-diol (ADIOL), and the anti-androgens cyproterone (CYP) and cyproterone acetate (CA) on plasma glucocorticoids and corticosterone binding globulin (CBG) in the immature male rat.

Both DHTP and ADIOL had dose-dependent inhibitory effects on the blood levels of corticosterone and CBG. However, DHTP inhibited plasma corticosterone levels relatively more than CBG, whereas ADIOL reduced both corticosterone and CBG to approximately the same extent; in both cases plasma corticosterone was reduced to a level which was approximately 40% of the control level. Cyproterone and CA reduced plasma corticosterone levels to a greater degree than was observed with DHTP or ADIOL. On the other hand, plasma CBG levels were either unaffected or only marginally decreased by anti-androgen treatment.

The conclusions made from this study were: (a) 5α reduced androgens have inhibitory effects on the pituitary-adrenal axis of the intact immature male rat. (b) the inhibitory effects of ADIOL on corticosterone levels can mainly be explained by its effects on CBG whereas DHTP appears to have an additional effect on adrenal secretion. (c) both anti-androgens exert a pronounced inhibitory influence on corticosterone levels via a mechanism which does not involve CBG.

INTRODUCTION

Several studies have shown that under certain conditions testosterone reduces the plasma levels of corticosterone in rats [1–3]. It is not known whether this effect is due to testosterone as such, or due to one of its 5α reduced metabolites (DHT or ADIOL). These effects of testosterone have been observed only in situations when levels of endogenous testosterone are low, as in the adult female rat or in the castrated adult male. Since most of the circulating corticosterone is bound to CBG and only a small proportion is in the form of free (biologically active) steroid, a reduction in plasma corticosterone levels may not always reflect an alteration of adrenal secretion but a change in its plasma binding. The one major study which has been concerned with the effects of exogenous androgens on plasma CBG has been limited to adult rats [3]. The purpose of the present study was to examine the effects of 5α reduced androgens (DHTP or ADIOL) as well as anti-androgens (CYP and CA) on plasma corticosterone in immature male rats, and to ascertain to what extent the observed effects can be attributed to alterations in the blood levels of CBG.

MATERIALS AND METHODS

Animals and hormone treatment. Male Sprague–Dawley rats, 21 days old were injected intramuscularly daily for ten days with increasing doses of DHTP, ADIOL, CYP or CA. Control groups were injected daily with the same vol. of oil vehicle. All groups were composed of five to six animals except in the control group of the ADIOL study which contained 14. After 10 days, all animals were killed between 8 am and 10 am by cervical dislocation and exsanguinated. The resulting plasma was aliquotted and stored at −20°C.

Measurement of plasma corticosterone. Adrenal secretion was monitored using the competitive protein binding method of Nugent and Mayes[4] with few modifications. The method utilises human CBG as the binding protein which is obtained from third trimester pregnancy plasma. In view of the high cross reactivity of the majority of the plasma glucocorticoids with this protein and since no chromatographic step was included, this method provided an index of total circulating glucocorticoids. However, since corticosterone is the major glucocorticoid in the rat further reference to this index is made in terms of corticosterone levels. The advantage of the above technique is that it provides a measure of the total secretion of glucocorticoids from the adrenal cortex and ignores more subtle changes in the relative quantities of the individual components of adrenal secretion.

In brief, corticosterone was extracted from small aliquots of plasma (10 μl) with dichloromethane (1.0 ml). After evaporation of the organic phase, the residue was redissolved in phosphate buffer (0.1 M,

pH 7.4, 0.1 ml). The binding assay was performed overnight at 4°C using florasil-stripped binding protein and [1,2,^{3}H]-cortisol as the competing tracer. The intrassay and interassay variation rarely exceeded 6% and 10% respectively. Blanks were at all times negligible and using a plasma vol. of 10 μl the limit of assay sensitivity was 62.5 ng/ml. In cases where this level was not exceeded, higher vols of plasma were processed.

Measurement of plasma CBG. Plasma CBG was measured using steady state gel electrophoresis. The details of this method as applied to rat plasma have been presented elsewhere [5]. Intra- and interassay variation were less than 8 and 10% respectively.

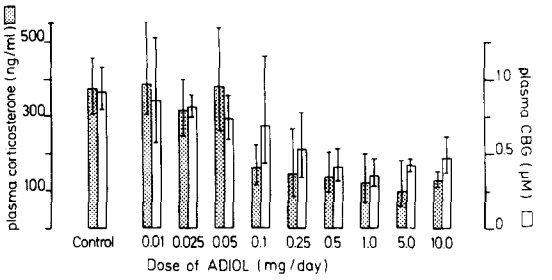

Fig. 2. Effects of increasing doses of 5α-androstan-3α, 17β-diol (ADIOL) on plasma corticosterone and CBG levels in the immature male rat. Columns and vertical bars represent geometric means with 95% confidence limits.

RESULTS

Comparisons between the different groups were tested using a one way analysis of variance after logarithmic transformation of the data. The geometric means and 95% confidence limits for the control and treatment groups are presented in Figs. 1–4.

Effects of DHTP and ADIOL

A daily dose of 50 μg/day DHTP significantly ($P < 0.05$) decreased plasma corticosterone values below control levels (Fig. 1). Higher doses exaggerated this decrease in a dose-dependent manner (100–500 μg/day: $P < 0.01$; 1000–10,000 μg/day: $P < 0.001$). The highest doses of DHTP (1000–10,000 μg/day) inhibited plasma corticosteroid levels to approximately 40% of the control level. The same steroid had similar but less pronounced effects on plasma CBG. Administration of 50–5000 μg/day significantly reduced plasma CBG to a level which was approximately 80% of the control value (50, 500, 1000 μg/day: $P < 0.05$; 100, 250, 5000 μg/day: $P < 0.01$).

In the case of ADIOL (Fig. 2) slightly larger doses were required to produce a significant reduction (250 μg/day and 100 μg/day for plasma CBG and corticosterone respectively). However, once this threshold was attained ADIOL was as effective as DHTP in suppressing plasma levels of corticosterone, giving a maximum inhibition of approximately 40% of control values ($P < 0.001$ in all cases above the dose levels 100 μg/day). ADIOL was more effective in suppressing plasma CBG levels than was DHTP. Plasma CBG after ADIOL treatment was significantly reduced at all doses above 100 μg/day ($P < 0.001$), and at the highest doses (1000–10,000 μg/day) CBG levels were reduced to approximately 40% of control values.

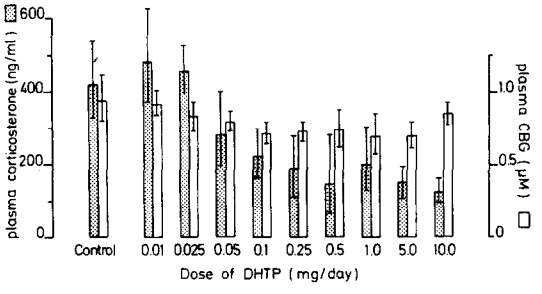

Fig. 1. Effects of increasing doses of dihydrotestosterone propionate (DHTP) on plasma corticosterone and CBG levels in the immature male rat. Columns and vertical bars represent geometric means with 95% confidence limits.

Effects of cyproterone and cyproterone acetate

Surprisingly, both anti-androgens had effects on plasma corticosterone which were similar to that of the androgens. All doses of CA above 0.5 mg/day caused a significant reduction in plasma corticosterone levels ($P < 0.001$ in all cases; Fig. 3). Larger quantities of the compound (2.5 mg/day) were necessary to elicit a significant decrease in plasma CBG which was subsequently reduced in a dose-dependent manner (2.5 mg: $P < 0.05$; 5.0 mg: $P < 0.01$; 10 mg: $P < 0.001$). The lowest levels of corticosterone and CBG attained by CA treatment were approximately 5% and 60% of the control level respectively.

Cyproterone significantly decreased plasma corticosterone levels below the level of the control population at doses of 2 mg ($P < 0.05$) and 5 mg/day ($P < 0.001$), without detectable effects on plasma CBG (Fig. 4).

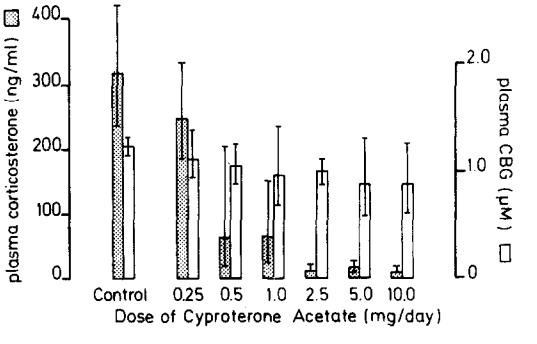

Fig. 3. Effects of increasing doses of cyproterone acetate on plasma corticosterone and CBG levels in the immature male rat. Columns and vertical bars represent geometric means with 95% confidence limits.

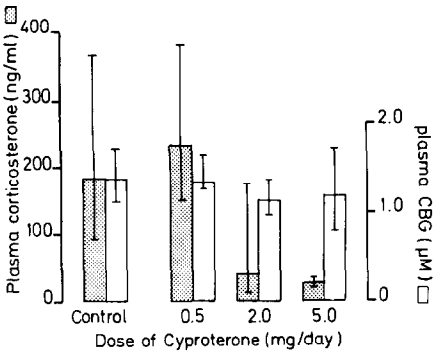

Fig. 4. Effects of increasing doses of cyproterone on plasma corticosterone and CBG levels in the immature male rat. Columns and vertical bars represent geometric means with 95% confidence limits.

DISCUSSION

Treatment of immature male rats with the 5α reduced androgens DHTP and ADIOL results in a dose-dependent decrease in plasma levels of corticosterone and CBG. In the case of DHTP, corticosterone levels were reduced twice as much as plasma CBG indicating that DHTP, in addition to its effect on the liver (CBG), also must have an additional effect on the adrenal secretion of glucocorticoids. In the case of ADIOL, CBG was reduced almost to the same extent as corticosterone suggesting that the effect of ADIOL on plasma corticosterone is mainly due to a reduction in CBG, and that this androgen may have much less pronounced direct effects on the pituitary-adrenal axis.

In the intact adult male rat, several workers have demonstrated that testosterone administration has little or no effect on either corticosterone or CBG [1–3, 6]. However, castrated adult males and intact adult females exhibit significantly elevated levels of corticosterone and CBG which are reduced to those of intact adult males by testosterone treatment [1–3]. The explanation may be that the normal endogenous levels of androgen in the adult male is already maximally suppressing corticosterone and CBG which cannot be reduced further by exogenous treatment. From these experiments, it is obvious that the immature intact male rats are responding like the adult male castrate and female to exogenous androgen administration.

Testosterone inhibits pituitary responsiveness to CRF *in vitro* [7]. This mechanism probably accounts for the major effects of androgen on adrenal secretion observed in the present study, especially those of DHTP. The fact that the rat adrenals contain specific intracellular androgen receptors identical to those in peripheral sex tissues [8], suggests that a direct action of testosterone and DHT on the adrenal cortex is also likely. However, from studies on the testicular feminized male (*tfm*) rats, we believe that the direct effects of androgen on adrenal secretion are stimulatory rather than inhibitory. The *tfm* rat has a normal plasma level of corticosterone but a greatly elevated level of CBG indicating a reduced level of free "active" corticosterone, and a reduced adrenal corticosterone production [9]. The marked enlargment of the adrenal glands in the *tfm* rat which are heavier than in the female of the species [10] is therefore probably a compensatory phenomenon secondary to the reduced quantities of corticosterone acting on the pituitary and inhibiting ACTH secretion. Adult female rats also possess elevated plasma levels of CBG but in their case the peripheral corticosterone levels are elevated to a comparable degree [3], suggesting that adrenal production is normal. However, it should also be noted that the adrenal androgen content of female rats does not differ from that of normal males [8] indicating that sufficient androgen is locally produced in the adrenal gland to exert an influence on adrenal secretion. In the case of the *tfm* rat the availability of androgen is not the problem. The absence of androgen receptors in the adrenal of these animals means that the androgens present in this organ are unable to exert any effects on adrenal function.

The antiandrogens CYP and CA have both been shown to cause decreased adrenal weight in male rats [11]. In the case of CA, this may not represent a direct effect on the adrenal glands since there is no suppression of adrenal gland weight by this compound when injected into hypophysectomised rats substituted with ACTH [12]. Moreover, the inhibitory influence of CA on plasma CBG cannot account for the marked reduction in glucocorticoid levels observed in the present study, as it can in the case of ADIOL. It thus appears that CA exerts its primary action on ACTH secretion, an effect which has been attributed to certain corticoid-like effects which this compound is believed to possess [11]. In confirmation of this statement, two groups of workers have demonstrated that CA therapy in human subjects causes a suppression of immunoreactive plasma ACTH [13, 14]. The effect of CYP in the present study indicates that this anti-androgen may also have a glucocorticoid-like effect on the pituitary [11]. Such effects would tend to obscure any manifestations of its direct antiandrogen influence on the adrenal. What is also of great interest is that both antiandrogens even in very large quantities have little or no effect on the circulating levels of CBG. Cyproterone acetate is known to have strong progestational side-effects whereas CYP is devoid of such activity [11]. The fact that CA and CYP have similar effects on corticosterone levels makes it therefore unlikely that this influence of CA on adrenal function is a consequence of its progestational properties.

The present study provides examples of three different ways hormones or drugs may exert their effects on the secretion of adrenal hormones at least with regard to the involvement of CBG: They may exert an effect primarily on the binding protein CBG and have less influence on the pituitary-adrenal axis (e.g.

ADIOL), they may exert an effect primarily on the pituitary-adrenal axis with little or no effect on the levels of the binding protein (e.g. CYP and CA) or they may have an effect which is directed simultaneously toward both sites (e.g. DHTP). Future studies on hormone and drug influences on the levels of circulating steroids should include an assessment of the appropriate plasma binding proteins. In this way, at least one of the potential sites of action of such compounds can be excluded from the overall analysis of the mechanisms of their effect.

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